10070791.DOC=070791
10/070791
JC13 Rec'd PCT/PTO 08 MAR 2002

CARBOCYCLIC NUCLEOSIDES AND PROCESS FOR OBTAINING SUCH

The invention relates to carbocyclic nucleoside analogues and their pharmaceutically acceptable salts, and to a method for the production of such, and to their use as anti-viral agents, amongst others.

Most antiviral compounds belong to the nucleoside field and the development of new modified nucleosides as antiviral agents is an active field of research. An object of the present invention is to provide an agent exhibiting pharmaceutical activity.

According to a first aspect of the present invention there is provided a carbocyclic nucleoside analogue as described in claims 1-14.

These carbocyclic nucleotides exhibit good pharmaceutical activity.

According to a second aspect of the present invention there is provided a process for providing these carbocyclic compounds and intermediates thereof according to claims 15-24.

According to a further aspect of the present invention there is provided a carbocyclic compound according to any of the claims 24-35.

According to a further aspect of the present invention there is provided a pharmaceutical composition and the use of such, according to the claims 36-39.

Further aspects of the invention are detailed in claims 40-43.

The inventors have developed an enantioselective approach toward the synthesis of (D)-cyclohexene nucleoside 4 (Figure 3) using R-(-)-carvone (1) as inexpensive starting material. A sequence of chemical transformations led to intermediate 2, possessing four chiral centers and, disregarding the protecting groups R_1-R_4, a plane of symmetry. Intermediate

2 allows for the synthesis of both the (D)- and the (L)-cyclohexene nucleosides 4 and 5, and 7 and 8, respectively.

The synthesis of (D)-adenine cyclohexene nucleoside 4 was accomplished by using a Mitsunobu type reaction on enol 3 to introduce the adenine base moiety on the cyclohexenyl ring. The corresponding guanine derivative 5 was synthesized in a similar way. Enol 3 was reacted with 2-amino-6-chloropurine in the presence of DEAD and triphenyl phosphine in dioxane to give 9 (Figure 4), which was converted to 5 by treatment with TFA-H_2O 3:1. Under these reaction conditions the two TBDMS protecting groups were simultaneously removed. The overall yield starting from 3 was 46%. Analytically pure 5 was obtained by reversed-phase HPLC purification.

The synthesis of the (L)-cyclohexene nucleosides 7 and 8 was carried out by protection of the C4-CH_2OH and C5-OH groups (2b), followed by conversion of the OR_2 and OR_4 groups into enol 6. This compound was then used for the introduction of the base moiety according to the same strategy as used for the (D)-series. Intermediate 2b (R_1 = R_3 = H; R_2 = R_4 = TBDMS) was converted to dibenzoate 10 (Figure 5) using standard reaction conditions (Bz_2O, DMAP, CH_2Cl_2, 98%). The equatorial C3-OTBDMS protecting group was selectively removed in the presence of the axial C1-OTBDMS to give 11 by using one equivalent of TBAF in THF at room temperature (74%). The selectivity of the desilylation reaction has been observed before[13c]. The C3 alcohol 11 was converted to the corresponding mesylate 12, the C1-OTBDMS group was removed using TBAF to give the alcohol 13 (96%), which was oxidized using PDC in CH_2Cl_2. This oxidation was accompanied by simultaneous elimination of the C3-OMs group to afford directly the desired enone 15 (68%). Stereoselective reduction of enone 15 using $NaBH_4$ in the presence of $CeCl_3.7H_2O$ in MeOH gave enol 6 (75%).

A Mitsunobu reaction was then applied for introduction of the base moiety (Figure 6). Upon reaction

of 6 with adenine in the presence of DEAD and PPh_3 in dioxane, the desired adenine derivative 16a was isolated in 40% yield, together with 15% of N7-isomer 16b (Figure 6). Finally, removal of the benzoyl protecting groups using K_2CO_3 in MeOH gave the (L)-adenine cyclohexene nucleoside 7 in 72% yield. The corresponding (L)-guanine nucleoside 8 was synthesized in an analogous way. The enol 6 was treated with 2-amino-6-chloropurine under the same reaction condition as described above for 9, and the obtained 6-chloropurine 17 was converted to the guanine derivative 18 using TFA-H_2O 3:1 (58% yield from 6). Final deprotection was carried out by heating 18 in a saturated solution of NH_3 in MeOH in a sealed vessel for 2 days and reversed-phase HPLC purification gave pure (L)-guanine cyclohexene nucleoside 8 in 73% yield.

Antiviral activity

The anti-herpesvirus activity of D-cyclohexene G and L-cyclohexene G and the respective adenine analogues was determined in human embryonic skin muscle fibroblast (E_6SM: HSV-1, HSV-2) and in human embryonic lung (HEL) cells [varicella-zoster virus (VZV), cytomegalovirus (CMV)] (Table I). The source of the viruses and the methodology used to monitor antiviral activity have been previously described (De Clercq, E., Descamps, J., Verhelst, G., Walker, R.T., Jones, A.S., Torrence, P.F., Shugar, D. Comparative efficacy of antiherpes drugs against different strains of herpes simplex virus. J.Infect. Dis. 1980, 141, 563-574; De Clercq, E., Hóly, A., Rosenberg, I., Sakuma, T., Balzarini, J., Maudgal, P.C. A novel selective broad-spectrum anti-DNA virus agent. Nature, 1986, 323, 464-467). The antiviral activity was compared with the activity of known and approved antiviral drugs from which two with a purine base moiety (acyclovir, ganciclovir) and two with a pyrimidine base moiety (brivudine, cidofovir).

D-cyclohexene G as well as L-cyclohexene G did

not show toxicity in four different cell lines (HeLa, Vero, E_6SM, HEL) (Table II), pointing to their selective antiviral mode of action, as reflected by the high selectivity index of the compounds (Table I). A salient feature is that the activity spectrum of both enantiomers is remarkably similar. Both compounds display the same activity against HSV-1 and HSV-2. Against VZV and CMV the potency of L-cyclohexene G is about 2-fold lower than that of D-cyclohexene G. Against HSV-1, the cyclohexene G nucleosides are as active as acyclovir and brivudin. Against HSV-2, their activity is very similar to that of acyclovir. The cyclohexene G nucleosides retain activity against the TK^- strains of HSV-1 and VZV, although the activity is reduced as compared to the activity against the wild type. The activity of D-cyclohexene G against TK^+ and TK^- VZV strains is higher than the respective activities of acyclovir and brivudin against these viruses. D-cyclohexene G has the same activity against CMV as ganciclovir. In conclusion the activity spectrum of the cyclohexene nucleosides of the present invention is very similar to that of the known antiviral compounds possesing a guanine base moiety (acyclovir, ganciclovir). Both the D- and the L-enantiomers of cyclohexene G are antivirally active. The high selectivity indexes observed for D- and L-cyclohexene G indicates the utility of these compounds against herpesvirus infections.

D-cyclohexene G as well as L-cyclohexene G exhibited potent and selective anti-herpes virus (HSV1, HSV2, VZV, CMV) activity. Their activity spectrum is comparable to that of the known antiviral drugs acyclovir and ganciclovir. D- and L-cyclohexene G represent a very potent antiviral nucleosides containing a six-membered carbohydrate mimic. In contrast to the nucleosides with a cyclohexane, pyranose or hexitol ring, the cyclohexene nucleosides have a very flexible conformation. The inventors theorize that this flexibility may be an important structural determinant for their potent antiviral activity.

Experimental (1)

General Methods

Melting points were determined in capillary tubes with a Büchi-Tottoli apparatus and are uncorrected. Ultraviolet spectra were recorded with a Philips PU 8740 UV/vis spectrophotometer. ^{1}H NMR and ^{13}C NMR were determined with a 200 MHz Varian Gemini apparatus with tetramethylsilane as internal standard for the ^{1}H NMR spectra and DMSO-d_6 (39.6 ppm) or $CDCl_3$ (76.9 ppm) for the ^{13}C NMR spectra (s = singlet, d = doublet, dd = double doublet, t = triplet, br s = broad singlet, br d = broad doublet, m = multiplet). Liquid secondary ion mass spectra (LSIMS) with Cs^+ as primary ion beam were recorded on a Kratos Concept IH (Kratos, Manchester, U.K.) mass spectrometer equipped with a MASPEC2 data system (Mass Spectrometry Services Ltd., Manchester, U.K.). Samples were directly dissolved in glycerol (gly)/thioglycerol (thgly)/*m*-nitrobenzyl alcohol (nba) and the secondary ions accelerated at 7kV. Scans were performed at 10 s/decade from *m/z* 1000 down to *m/z* 50. Precoated Machery-Nagel Alugram SIL G/UV_{254} plates were used for TLC (in solvent systems: A CH_2Cl_2-MeOH 98:2, B CH_2Cl_2-MeOH 9:1, C CH_2Cl_2-EtOAc 4:1); the spots were examined with UV light and sulfuric acid/anisaldehyde spray. Elemental analyses were done at the University of Konstanz, Germany.

9-[(1*S*,4*R*,5*S*)-5-(tert-butyldimethylsilyloxy)-4-(tert-butyldimethylsilyloxymethyl)-2-cyclohexenyl]-2-amino-6-chloropurine (9)

To a mixture of 3 (130 mg, 0.35 mmol), 2-amino-6-chloropurine (119 mg, 0.70 mmol) and PPh_3 (184 mg, 0.70 mmol) in dry dioxane (7 mL) under N_2 at room temperature was added a solution of DEAD (110 µL, 0.70 mmol) in dry dioxane (3 mL) over a period of 1.5 hr. The reaction mixture was stirred at room temperature for two days and concentrated. The residue was chromatographed on silica

gel (CH_2Cl_2-MeOH 50:1, then 20:1) to yield crude 9 (170 mg) as a yellow foam: ^1H NMR ($CDCl_3$) δ -0.10 (s, 3H), -0.04 (s, 3H), 0.07 (s, 6H), 0.82 (s, 9H), 0.90 (s, 9H), 2.04 (t, 2H, J = 5.6 Hz), 2.27 (m, 1H), 3.66 (dd, 1H, J = 9.9, 55.1 Hz), 3.77 (dd, 1H, J = 9.9, 4.4 Hz), 3.98 (m, 1H), 5.21 (m, 1H), 5.43 (s, 2H, NH_2), 5.79 (dm, 1H, J = 9.9 Hz), 6.00 (dm, 1H, J = 9.9 Hz), 7.79 (s, 1H); ^{13}C NMR ($CDCl_3$) δ -5.6 (q), -5.5 (q), -5.1 (q), -4.8 (q), 17.8 (s), 18.2 (s), 25.6 (q), 25.8 (q), 36.0 (t), 46.9 (d), 1049.2 (d), 62.9 (t), 64.6 (d), 124.4 (d), 125.4 (s), 134.4 (d), 141.3 (d), 151.1 (s), 153.3 (s), 159.1 (s);

9-[(1*S*,4*R*,5*S*)-5-hydroxy-4-hydroxymethyl-2-cyclohexenyl]guanine (5)

Crude 9 (170 mg) was treated with TFA-H_2O (3:1, 10 mL) at room temperature for two days. The reaction mixture was concentrated and co-evaporated with toluene (2x). The residue was chromatographed on silica gel (CH_2Cl_2-MeOH 10:1, then 1:1) to afford 5 (45 mg, 46% overall yield starting from 3): Mp >230 °C; UV λ_{max} (MeOH) = 253 nm, ^1H NMR (CD_3OD) δ 1.94-2.27 (m, 3H), 3.77 (d, 2H, J = 4.7 Hz), 3.85 (m, 1H), 5.17 (m, 1H), 5.88 (dm, 1H, J = 10.2 Hz), 6.09 (dm, 1H, J = 10.2 Hz), 7.73 (s, 1H); ^{13}C NMR (CD_3OD) δ 37.1 (t), 47.7 (d), 50.6 (d), 63.1 (t), 64.8 (d), 125.8 (d), 135.4 (d), 138.5 (d); LISMS (THGLY/NBA) 278 $(M+H)^+$; HRMS calcd for $C_{12}H_{16}N_5O_3$ $(M+H)^+$ 278.1253, found 278.1270; Anal. Calcd for $C_{12}H_{15}N_5O_3.0.77H_2O$: C 49.49, H 5.73, N 24.05; Found: C 49.45, H 5.55, N 24.22.

(1*S*,2*S*,3*R*,5*R*)-3-Benzoyl-2-benzoylmethyl-1,5-di(*tert*-butyldimethylsilyloxy)-cyclohexane (10)

To a solution of 2b (2.2 g, 5.64 mmol) in dry dichloromethane (20 mL) at 0 °C under N_2 was added DMAP (3.44 g, 28.2 mmol, 5 eq) and Bz_2O (3.83 g, 16.92 mmol, 3 eq) sequentially and in portions. After stirring at 0 °C for 2 hr, the reaction was quenched with ice. The

reaction mixture was poured into CH_2Cl_2 (250 ml) and washed with water and brine, dried over Na_2SO_4 and concentrated. The crude product was chromatographed on silica gel (*n*-hexane-EtOAc 10:1) to yield 10 (3.3 g, 98%) as a light yellow oil.

^1H NMR ($CDCl_3$) δ 0.03 (s, 3H), 0.06 (s, 3H), 0.13 (s, 3H), 0.17 (s, 3H), 0.89 (s, 9H), 1.01 (s, 9H), 1.58 (m, 2H), 2.09 (m, 2H), 2.37 (m, 1H), 4.29 (br-s, 1H), 4.40 (td, 1H, J = 10.3, 4.0 Hz), 4.49 (dd, 1H, J = 11.4, 2.0 Hz), 4.61 (dd, 1H, J = 11.4, 2.2 Hz), 5.66 (td, 1H, J = 11.0, 4.6 Hz), 7.37 - 7.44 (m, 4H), 7.49 - 7.59 (m, 2H), 8.02 (m, 4H);

^{13}C NMR ($CDCl_3$) δ -5.3 (q), -5.2 (q), -5.1 (q), -4.5 (q), 17.8 (s), 25.6 (q), 25.7 (q), 38.2 (t), 42.4 (t), 49.5 (d), 60.0 (t), 65.2 (d), 66.4 (d), 68.5 (d), 128.3 (d), 129.6 (d), 130.3 (s), 130.4 (s), 132.8 (d), 165.6 (s), 166.5 (s);

(1*S*,2*S*,3*R*,5*S*)-3-Benzoyl-2-benzoylmethyl-5-(*tert*-butyldimethylsilyloxy)-cyclohexanol (11)

A solution of TBAF 1M in THF (5.38 ml, 5.38 mmol) was added slowly to a solution of 10 (3.23 g, 5.38 mmol) in THF (50 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 2 hr and further at room temperature for 3 hr. Ice was added and the reaction mixture was poured into EtOAc (300 ml) which was washed with NH_4Cl solution, water and brine, dried over Na_2SO_4 and concentrated. The crude product was purified on silica gel (*n*-hexane-EtOAc 5:1 then 1:1) to yield 11 (1.93 g, 74%) as a white foam.

^1H NMR ($CDCl_3$) δ 0.02 (s, 3H), 0.09 (s, 3H), 0.81 (s, 9H), 1.50 - 1.63 (m, 2H), 1.96 (m, 1H), 2.13 (m, 1H), 2.32 (m, 1H), 3.24 (d, 1H, J = 4.8 Hz, -OH), 4.04 (m, 1H), 4.25 (m, 1H), 4.33 (dd, 1H, J = 11.4, 2.2 Hz), 5.04 (dd, 1H, J = 11.4, 2.2 Hz), 5.60 (td, 1H, J = 11.0, 4.5 Hz), 7.36 - 7.60 (m, 6H), 8.06 (m, 4H);

^{13}C NMR ($CDCl_3$) δ -5.2 (q), 17.6 (s), 25.4 (q), 38.2 (t),

40.8 (t), 50.4 (d), 59.8 (t), 64.1 (d), 66.1 (d), 68.4 (d), 128.4 (d), 129.6 (d), 129.8 (d), 130.4 (s), 133.0 (d), 133.2 (d), 165.6 (s), 167.6 (s);

LISMS (THGLY/TFA): 485 $(M+H)^+$; HRMS calcd for $C_{27}H_{37}O_6Si$ $(M+H)^+$ 485.2359, found 485.2376.

(1*S*,2*S*,3*R*,5*S*)-3-Benzoyl-2-benzoylmethyl-5-(*tert*-butyldimethylsilyloxy)-1-methanesulfonyloxy-cyclohexane (12)

To a solution of **11** (1.90 g, 3.92 mmol) in dry dichloromethane (20 mL) at 0 °C under N_2 was added slowly triethylamine (2.71 ml, 19.6 mmol, 5 eq) and MsCl (456 µl, 5.89 mmol, 1.5 eq) sequentially. After stirring at 0 °C for 1 hr, the reaction was quenched with ice. The reaction mixture was poured into CH_2Cl_2 (250 ml) and washed with a saturated NH_4Cl solution, water and brine, dried over Na_2SO_4 and concentrated. The residue was chromatographed on silica gel (*n*-hexane-EtOAc 1:1) to afford **12** (2.17 g, 98%) as a white foam.

^1H NMR ($CDCl_3$) δ 0.13 (s, 3H), 0.15 (s, 3H), 0.96 (s, 9H), 1.62 (td, 1H, J = 12.0, 2.0 Hz), 1.85 (td, 1H, J = 12.0, 2.0 Hz), 2.26 - 2.58 (m, 3H), 2.98 (s, 3H), 4.34 (m, 1H), 4.50 (dd, 1H, J = 11.4, 2.0 Hz), 4.60 (dd, 1H, J = 11.4, 2.0 Hz), 5.28 (td, 1H, J = 11.1, 4.8 Hz), 5.69 (td, 1H, J = 11.0, 4.8 Hz), 7.42 (m, 4H), 7.57 (m, 2H), 8.03 (m, 4H);

^{13}C NMR ($CDCl_3$) δ -5.3 (q), -5.2 (q), 17.8 (s), 25.5 (q), 37.8 (t), 38.1 (q), 39.9 (t), 46.7 (d), 58.9 (t), 65.9 (d), 67.8 (d), 75.9 (d), 128.5 (d), 129.6 (d), 129.9 (s), 133.1 (d), 165.4 (s), 166.3 (s);

LISMS (THGLY/GLY): 563 $(M+H)^+$; HRMS calcd for $C_{28}H_{39}O_8SSi$ $(M+H)^+$563.2135, found 563.2188.

(1*R*,3*S*,4*S*,5*R*)-5-Benzoyl-4-benzoylmethyl-3-methanesulfonyloxy-cyclohexanol (13)

To a solution of **12** (2.15 g, 3.82 mmol) in THF (50 mL) at room temperature was added slowly a 1 M

solution of TBAF (7.64 ml, 7.64 mmol, 2 eq) in THF. The reaction was stirred at room temperature for 2.5 hr and quenched with ice. After standard work-up and purification on silica gel (*n*-hexane-EtOAc 1:1), **13** (1.55 5g, 86%) was obtained as a white foam.

^{1}H NMR ($CDCl_3$) δ 1.71 (td, 1H, *J* = 12.1, 2.2 Hz), 1.90 (td, 1H, *J* = 12.2, 2.3 Hz), 2.29 - 2.65 (m, 4H), 3.00 (s, 3H), 4.41 (m, 1H), 4.52 (dd, 1H, *J* = 11.7, 2.8 Hz), 4.61 (dd, 1H, *J* = 11.7, 2.8 Hz), 5.27 (td, 1H, *J* = 10.6, 4.8 10Hz), 5.65 (td, 1H, *J* = 10.6, 4.7 Hz), 7.42 (m, 4H), 7.55 (m, 4H), 8.02 (m, 4H);

^{13}C NMR ($CDCl_3$) δ 37.3 (t), 38.2 (q), 39.0 (t), 46.8 (d), 59.3 (t), 65.1 (d), 67.9 (d), 75.8 (d), 128.5 (d), 129.7 (d), 133.2 (d), 133.3 (d), 165.6 (s), 166.4 (s);

15 LISMS (THGLY/TFA): 449 $(M+H)^+$; HRMS calcd for $C_{22}H_{25}O_6S$ $(M+H)^+$ 449.1270, found 449.1244.

(4*S*,5*R*)-5-Benzoyl-4-benzoylmethyl-cyclohex-2-en-1-one (15)

20 A mixture of **13** (500 mg, 1.12 mmol) and PDC (2.1 g, 5.60 mmol, 5 eq) in dry CH_2Cl_2 (30 mL) was stirred vigorously at room temperature for 24 h. The reaction mixture was filtered through Celite® and washed with CH_2Cl_2. The filtrate was concentrated and the residue was 25chromatoghraphed on silica gel (*n*-hexane-EtOAc 2:1, then 1:2) to yield starting material **13** (100 mg, 20 %) and enone **15** (267 mg, 68%) as a light yellow oil.

^{1}H NMR ($CDCl_3$) δ 2.73 (dd, 1H, *J* = 16.5, 8.8 Hz), 3.10 (dd, 1H, *J* = 16.5, 4.4 Hz), 3.27 (m, 1H), 4.50 (dd, 1H, *J* 30= 11.3, 4.7 Hz), 4.66 (dd, 1H, *J* = 11.3, 5.5 Hz), 5.66 (ddd, 1H, *J* = 8.8, 7.3, 4.4 Hz), 6.26 (dd, 1H, *J* = 10.2, 2.2 Hz), 6.96 (dd, 1H, *J* = 10.2, 3.3 Hz), 7.40 - 7.63 (m, 6H), 8.01 (m, 4H);

^{13}C NMR ($CDCl_3$) δ 41.3 (d), 42.1 (t), 63.2 (t), 70.0 (d), 35128.6 (d), 129.5 (2s), 129.7 (d), 129.8 (d), 131.4 (d), 133.5 (d), 146.5 (d), 165.5 (s), 166.4 (s), 195.8 (s);

(1S,4S,5R)-5-Benzoyl-4-benzoylmethyl-cyclohex-2-en-1-ol (6)

To a solution of 15 (267 mg, 0.76 mmol) in MeOH (10 mL) at room temperature under N_2 was added $CeCl_3.7H_2O$ (426 mg, 1.14 mmol, 1.5 eq). The mixture was stirred for 0.5 h and a clear solution was obtained. $NaBH_4$ (35 mg, 0.91 mmol, 1.2 eq) was added in portions and H_2 evolved. The reaction mixture was stirred for 1 h and quenched with ice. The mixture was stirred for 15 min and concentrated. The residue was distributed into EtOAc, washed with H_2O and brine, dried over Na_2SO_4 and concentrated. The residue was chromatographed on silica gel (*n*-hexane-EtOAc 10:1) to give 6 (200 mg, 75%) as a light yellow oil.

^1H NMR ($CDCl_3$) δ 1.77 (d, 1H, J = 7.2 Hz), 1.93 (ddd, 1H, J = 12.1, 10.2, 8.0 Hz), 2.54 (ddd, 1H, J = 12.1, 5.8, 3.3 Hz), 3.00 (m, 1H), 4.32 (dd, 1H, J = 11.4, 5.5 Hz), 4.44 (dd, 1H, J = 11.4, 5.5 Hz), 4.50 (m, 1H), 5.35 (ddd, 1H, J = 10.2, 7.3, 2.9 Hz), 5.78 (dt, 1H, J = 10.2, 1.8 Hz), 5.97 (dt, 1H, J = 10.2, 2.5 Hz), 7.34 - 7.60 (m, 6H), 8.00 (m, 4H);

^{13}C NMR ($CDCl_3$) δ 36.6 (t), 40.9 (d), 46.6 (t), 65.8 (d), 69.9 (d), 126.6 (d), 128.4 (d), 128.5 (d), 129.7 (d), 129.8 (s), 130.9 (s), 132.7 (d), 133.1 (d), 133.2 (d), 166.0 (s), 166.5 (s);

LISMS (THGLY/TFA): 353 $(M+H)^+$; HRMS calcd for $C_{21}H_{21}O_5$ $(M+H)^+$ 353.1389, found 353.1440.

9-[(1R,4S,5R)-5-Benzoyl-4-benzoylmethyl-2-cyclohexenyl]adenine (16a)

To a mixture of 6 (65 mg, 0.18 mmol), adenine (48 mg, 0.36 mmol, 2 eq) and PPh_3 (94 mg, 0.36 mmol, 2 eq) in dry dioxane (4 mL) under N_2 at room temperature was added a solution of DEAD (56 µL, 0.36 mmol, 2 eq) in dry dioxane (3 mL) over a period of 1 hr. The reaction mixture was stirred at room temperature overnight and concentrated. The residue was chromatographed on silica

gel (CH_2Cl_2-MeOH 50:1, 20:1, 10:1) to yield 16a (33 mg, 40%) as a white solid.

UV λ_{max} (MeOH): 231 and 263 nm.

^1H NMR ($CDCl_3$) δ 2.48 (ddd, 1H, J = 13.6, 8.3, 5.8 Hz), 2.57 (ddd, 1H, J = 13.6, 6.0, 3.2 Hz), 4.50 (dd, 1H, J = 10.4, 5.0 Hz), 4.63 (dd, 1H, J = 10.4, 6.1 Hz), 5.53 (m, 2H), 5.92 (s, 2H), 6.09 (dm, 1H, J = 10.0 Hz), 6.17 (dm, 1H, J = 10.0 Hz), 7.41 (m, 4H), 7.57 (m, 2H), 7.86 (s, 1H), 8.04 (m, 4H), 8.35 (s, 1H);

^{13}C NMR ($CDCl_3$) δ 32.4 (t), 40.6 (d), 48.7 (d), 64.3 (t), 68.2 (d), 120.1 (s), 126.8 (d), 128.5 (d), 128.6 (d), 129.6 (d), 129.7 (d), 131.0 (d), 133.4 (d), 138.8 (d), 149.8 (s), 153.1 (d), 155.8 (s), 165.8 (s), 166.5 (s);

LISMS (THGLY/NBA): 470 $(M+H)^+$; HRMS calcd for $C_{26}H_{24}N_5O_4$ $(M+H)^+$ 470.1828, found 470.1845.

9-[(1*R*,4*S*,5*R*)-5-hydroxy-4-hydroxymethyl-2-cyclohexenyl]adenine (7)

Compound 16a (33 mg, 0.07 mmol) was treated with anhydrous K_2CO_3 (100 mg) in MeOH (3 mL) at room temperature for 3 hr. Small portion of silica gel was added to the reaction mixture and the solvent was evaporated. The residue was chromatographed on silica gel (CH_2Cl_2-MeOH 10:1, 1:1) to give 7 (14 mg, 77%).

^1H NMR (CD_3OD) δ 2.02 - 2.32 (m, 3H), 3.79 - 3.90 (m, 3H), 5.35 (m, 1H), 5.93 (dm, 1H, J = 9.9 Hz), 6.15 (dm, 1H, J = 9.9 Hz), 8.09 (s, 1H), 8.21 (s, 1H);

^{13}C NMR (CD_3OD) δ 37.2 (t), 47.7 (d), 51.1 (d), 63.0 (t), 64.7 (d), 120.6 (s), 125.4 (d), 136.0 (d), 141.6 (d), 150.3 (s), 153.8 (d), 157.5 (s);

LISMS (THGLY/TFA): 262 $(M+H)^+$; HRMS calcd for $C_{12}H_{16}N_5O_2$ $(M+H)^+$ 262.1304, found 262.1323.

9-[(1*R*,4*S*,5*R*)-5-Benzoyl-4-benzoylmethyl-2-cyclohexenyl]guanine (18)

Compound 6 (160 mg, 0.45 mmol) was treated with 2-amino-6-chloropurine (153 mg, 0.90 mmol, 2 eq) in the

presence of PPh_3 (235 mg, 0.90 mmol, 2 eq) and DEAD (140 µl, 0.90 mmol, 2 eq) in dry dioxane (12 mL) at room temperature overnight. After concentration and purification on silica gel (CH_2Cl_2-EtOAc 1:1), crude 17 (500 mg) was obtained, which was treated with CF_3COOH/H_2O (3:1, 12 mL) at room temperature for 2 days. The reaction mixture was concentrated and coevaporated with toluene. The residue was purified on silica gel (CH_2Cl_2-MeOH 20:1) to yield 18 (126 mg, 58% over two steps) as a white solid.

UV λ_{max} (MeOH): 251 and 256 nm.

^{1}H NMR (500 MHz, DMSO-d_6) δ 2.30 (ddd, 1H, J = 13.6, 8.3, 5.9 Hz), 2.42 (ddd, 1H, J = 13.6, 6.4, 3.2 Hz), 3.00 (m, 1H), 4.52 (m, 2H), 5.17 (m, 1H), 5.37 (m, 1H), 6.03 (dm, 1H, J = 10.2 Hz), 6.11 (dm, 1H, J = 10.2 Hz), 6.45 (s, 2H), 7.51 (m, 4H), 7.66 (m, 2H), 7.69 (s, 1H), 7.95 (m, 4H), 10.61 (s, 1H);

^{13}C NMR (DMSO-d_6) δ 31.4 (t), 40.0 (d, overlapped with DMSO-d_6 peak), 47.9 (d), 64.4 (t), 68.5 (d), 116.9 (s), 127.0 (d), 128.9 (d), 129.3 (d), 129.4 (d), 130.2 (d), 133.6 (d), 135.7 (d), 150.9 (s), 153.8 (s), 156.9 (s), 165.3 (s), 165.8 (s);

LISMS (THGLY/GLY): 486 $(M+H)^+$; HRMS calcd for $C_{26}H_{24}N_5O_5$ $(M+H)^+$ 486.1777, found 486.1816;

UV (MeOH): 231, 256.

9-[(1*R*,4*S*,5*R*)-5-Hydroxy-4-hydroxymethyl-2-cyclohexenyl]guanine (8)

A mixture of 18 (85 mg) in an ammonium MeOH solution (75 mL) was sealed and heated at 80 °C for 2 days. After cooling to room temperature, the mixture was concentrated and the residue was purified by reverse HPLC (4% CH_3CN in water) to afford 8 (36 mg, 75%) as a white powder.

Mp: 255 °C (decomp.)

UV λ_{max} (MeOH) = 254 nm.

^{1}H NMR (500 HMz, DMSO-d_6) δ 1.85 (m, 1H), 1.98 (m, 1H),

2.11 (m, 1H), 3.54 (dd, 1H, J = 10.3, 5.5 Hz), 3.60 (dd, 1H, J = 10.3, 4.8 Hz), 3.70 (m, 1H), 4.68 (br-s, 1H, -OH), 4.75 (br-s, 1H, -OH), 4.99 (m, 1H), 5.77 (dm, 1H, *J* = 9.8 Hz), 5.97 (dm, 1H, *J* = 9.8 Hz), 6.57 (s, 2H, $-NH_2$), 57.50 (s, 1H), 10.8 (br-s, 1H, -NH);

^{13}C NMR (125 MHz, DMSO-d_6) δ 35.9 (t), 46.4 (d), 48.2 (d), 61.5 (t), 62.7 (d), 116.9 (s), 124.8 (d), 133.7 (d), 135.6 (d), 150.8 (s), 154.1 (s), 157.6 (s);

LISMS (THGLY/NBA) 278 $(M+H)^+$; HRMS calcd for $C_{12}H_{16}N_5O_3$ $(M+H)^+$ 278.1253, found 278.1247; Anal. Calcd for $C_{12}H_{15}N_5O_3.1.5H_2O$: C 47.35, H 5.96, N 23.03; Found: C 47.46, H 5.64, N 22.87.

As detailed above, the inventors have developed an enantioselective approach to the synthesis of six-membered carbocyclic nucleosides of type 2b (R = OH) starting from (*R*)-(-)-carvone (4, Figure 7, corresponding substantially with Figure 1). A key step involving hydroboration of the exo double bond of cyclohexene 6b to afford hydroxymethyl substituted 7b with the correct stereochemistry at C4. Precursor 6a provided an ideal starting material for the synthesis of 3 as it had (1) a protected hydroxyl group at C3, (2) a protected hydroxyl substituent at C1, which at a final stage can be used to introduce a base moiety with retention of the configuration using Pd-chemistry, and (3) a free hydroxyl group at C5, which could be used to introduce the double bond.

The most straightforward approach seemed to introduce the C5-C6 double bond *via* conversion of the OH at C5 into a suitable leaving group, followed by a regioselective elimination. The latter might be achieved *via* a E_2-type elimination reaction by treatment with base, which requires a neighbouring hydrogen *trans* to the leaving group, only available on C6. In order to explore this strategy, alcohol 6a was converted into diol 7a *via* hydroboration using 9-BBN in THF. The reaction gave 7a as the major isomer, together with a small amount of epimer

8a. The β-stereochemistry at C4 was easily established by NMR spectrometry. Selective protection of the primary hydroxyl group of 7a (TBDMSCl, imidazole, DMF) gave 9 (Figure 8) and the leaving group was introduced (MsCl, Et_3N, dichloromethane) to give 10. However, upon treatment of mesylate 10 with DBU in toluene, cyclohexene 11 was not formed. More vigorous reaction conditions (KOH, H_2O-THF),[5] likewise, failed to yield the unsaturated compound 11. Direct elimination of the 5-OH of 9 under Mitsunobu conditions (DEAD, PPh_3, THF)[6] was also unsuccessful. 9 was converted into the β-iodide 12 (I_2, PPh_3, imidazole, toluene), with inversion of the stereochemistry at C5, followed by treatment with DBU in refluxing toluene. This reaction resulted in an inseparable mixture (yield 68%) of cyclohexenes 11 and 13 in a 1:2.3 ratio, respectively, in favour of the undesired regioisomer.

The inventors also investigated a different synthetic strategy, i.e. the construction of an allylic acetate of type A or B (Figure 1) as intermediate for the Pd coupling reaction to introduce the base moiety.

Diol 14 (Figure 9) was protected as cyclic acetal 15 (2,2-dimethoxypropane, PPTS, acetone-THF), the Bn group was removed (10% Pd on carbon, $HCOONH_4$, MeOH, reflux) to give alcohol 16, and oxidation of the C5-OH (PDC, dichloromethane) provided ketone 17. Cleavage of the TBDMS ether using tetrabutylammonium fluoride (TBAF) in THF led mainly to diol 18. However, under neutral reaction conditions (KF, 18-crown-6, THF) the desired enone 19 was isolated in 62% yield; the β-hydroxy ketone intermediate 20 could not be detected. The critical reduction of enone 19 to the corresponding allylic alcohol 22 with β-oriented OH at C5 proved to be problematic, leading almost exclusively to the α-isomer 21 under the applied reaction conditions ($NaBH_4$, $CeCl_3.7H_2O$, MeOH and 9-BBN, THF). In an attempt to invert the stereochemistry at C5 of α-alcohol 21, the latter was

subjected to a Mitsunobu type reaction (DEAD, PPh_3, AcOH), but the desired β-acetate 23 was not formed. However, compound 21 might be used to synthesize the α-analogue of the aforementioned cyclohexene nucleoside, interesting for as well conformational analysis as for determination of antiviral activity.

The intended Pd coupling reaction was investigated on the α-acetate 24, easily prepared from 21 (Ac_2O, DMAP, dichloromethane). When 24 was treated with the anion (NaH) of adenine in the presence of tetrakis(triphenylphosphine)palladium(0) in DMF-THF, only 24 was recovered and no trace of the 1α-adenine 25 could be detected. Reasoning that this failure might be due to the rigidity of the cyclic acetal present, 24 was treated with PPTS in MeOH to give diol 26, which was then converted into the corresponding dibenzoate 27 (Bz_2O, DMAP, dichloromethane). However, upon subjection of 27 to the same reaction conditions for coupling as applied above to 24, the expected 1α-adenine product 28 could not be isolated.

The above failure having exhausted the possibilities of the Pd coupling strategy, the most reliable alternative (for the introduction of the base moiety seemed) a Mitsunobu reaction was utilized, i.e. by substitution with inversion of the configuration of an α-oriented hydroxyl group at C1. Therefore the inventors had to synthesize an appropriately protected precursor 7c. Epoxide 5b (Figure 7, R_1 = Bn) was converted into 6c under the reported conditions (LiTMP and Et_2AlCl in benzene-toluene 1:1) in 79% yield. Hydroboration of 6c with 9-BBN in THF afforded 7c as major isomer (74%), together with its epimer 8c (20%). Similar to configurational assignment of 7a and 7b, the β-stereochemistry at C4 of 7c was established by ^{1}H-NMR. The primary hydroxyl group of 7c was selectively protected using 1.2 equivalents of TBDMSCl and imidazole in DMF t_e

2ve 29 (70%, Figure 10), followed by conversion of the free alcohol at C5 into the corresponding mesylate 30 by treatment with MsCl and Et_3N in dichloromethane. Hydrogenolytic cleavage of the benzyl ether at C1 using 520% $Pd(OH)_2$ on carbon in the presence of cyclohexene in MeOH gave 31 in low yield (21%), which could be improved to 76% by the use of 10% Pd on carbon and $HCOONH_4$ in refluxing MeOH. Oxidation of alcohol 31 using PDC in dichloromethane gave a mixture of ketone 32 and enone 33 10in a combined yield of 39%. However, using MnO_2 in dichloromethane, an incomplete but clean reaction took place. The ketone 32 was not isolated and enone 33 was obtained in 48% yield and recovered 31 (47%) could be recycled. Finally, enone 33 was reduced using $NaBH_4$ in the 15presence of $CeCl_3.7H_2O$ in MeOH and gave the desired α-alcohol 34 as a single isomer in almost quantitative yield. The stereochemistry of 34 was confirmed by ^1H NMR spectral data. In $CDCl_3$ conformation A (Figure 2), with the three substituents in a pseudoaxial position, 20predominates due to intramolecular hydrogen bonding between the C1-OH and C3-OTBDMS groups, while in DMSO-d_6 it adopts conformation B. This reflects the much lower axial-equatorial energy differences in cyclohexenes as compared to the corresponding cyclohexanes.

25 With intermediate 34 in hand, the base moiety (adenine) was introduced under Mitsunobu reaction conditions. Upon treatment of 34 with adenine in the presence of DEAD and PPh_3 in dioxane at room temperature for 1 day, 35a was isolated in 66% yield, together with 3017% of its N_7-isomer 35b (Figure 11). Complete deprotection of 35a using TBAF in THF at room temperature afforded the desired cyclohexene carbocyclic nucleoside 36 in almost quantitative yield. However, the compound was contaminated with tetrabutylammonium salts which 35could not be removed by standard chromatographic techniques. Recently Parlow et al. described a work-up procedure to remove tetrabutylammonium salts by the

direct addition to the reaction mixture of mixed ion-exchange resins Amberlite® 15 and Amberlite® 15 in the Ca^{2+} form. Applied to the above TBAF reaction a complex mixture was obtained, giving 36 in low yield. In order to avoid the use of TBAF, Megron's method (Megron, G.; Vasquezy, F.; Galderon, G.; Cruz, R.; Gavino, R.; Islas, G. Synth. Commun. 1998, 26(16), 3021-3027) was used: compound 35a was treated with potassium *tert*-butoxide in DMF at room temperature. However, only a complex, reaction mixture was obtained, due to the strong basic character of the reaction conditions. Finally 35a was treated with a 3:1 mixture of TFA and H_2O at room temperature, which smoothly gave 36 in 54% overall yield starting from 34. According to our experience, this is the best procedure to cleave TBDMS ethers of this type of compound. Finally, 36 was purified by reversed-phase HPLC for analysis and determination of biological activity.

The above intermediate 36 (Figure 11) gave the inventors the opportunity to obtain as yet 2a (B = adenine) in enantiopure form via reduction of the double bond. Thus, 36 was hydrogenated using H2 under atmospheric pressure in the presence 10% Pd on carbon in MeOH at room temperature to afford D-2a in 75% yield. The spectral data of D-2a were superimposable with those of a DL mixture of 2a. The enantiomeric purity of D-2a was examined by HPLC on a chiral column. The separation of a DL mixture of 2a together with the HPLC profile of D-2a synthesized by the above approach is depicted in reference 3c. Its enantiomeric purity proved 99%, at the same time establishing the high enantiomeric purity of 36.

The inventors have developed an enantioselective approach towards the synthesis of cyclohexene carbocyclic nucleosides starting from (*R*)-carvone 4. The synthetic methodology makes use of a Mitsunobu reaction as the key step to introduce the heterocyclic base moiety. The reaction proved to be efficient as well as chemo- and stereoselective, while

the commonly applied palladium-mediated coupling strategy was unsuccessful. ^1H NMR and computation results show that in solution the synthesized adenine derivative 36 exists predominantly in a $^3H_2$ half-chair conformation with the adenine base orienting in a pseudoaxial position. The energy difference between $^3H_2$ and $^2H_3$ is, however, low. This compound may therefore be considered as a good mimic of a furanose nucleoside, showing two low energy conformations with a preference for the "3'-endo conformation". This is also the preferred conformation of a hexitol nucleoside, in the $^1C_4$ conformation. Moreover, the inventors theorize that the easy interconversion among both conformers might be a factor for antiviral activity.

Experimental (2)

(1*R*,3*S*,5*R*)-5-Benzyloxy-3-(*tert*-butyldimethylsilyloxy)-2-methylenecyclohexanol (6c)

A solution of 2,2,6,6-tetramethylpiperidine (TMP, 27.3 mL, 162 mmol) in dry benzene (80 mL) and dry toluene (80 mL) was cooled to 0 °C under N_2 and a solution of *n*-BuLi in hexane (1.6 M, 64.8 mL, 162 mmol) was added dropwise. The resulting mixture was stirred at 0 °C for 10 min and a solution of Et_2AlCl (1.8 M, 90 mL, 162 mmol) in toluene was slowly added over a period of 1 hr. The reaction was stirred for an additional 30 min. A solution of 5b (14.1 g, 40.5 mmol) in benzene (30 mL) was added slowly. The reaction mixture was stirred at 0 °C for 3 h, then poured into an ice-cold NH_4Cl solution (300 mL). A 3 N HCl solution was added until a clear emulsion was obtained. The layers were separated and the aqueous layer was extracted with EtOAc (3x). The combined organic layers were washed with H_2O and brine, dried over Na_2SO_4 and concentrated. The residue was chromatographed on silica gel (*n*-hexane-EtOAc 10:1) to give 6c (10.2 g, 71%) as a light-yellow oil: ^1H NMR ($CDCl_3$) δ 0.09 (s, 6H), 0.92

(s, 9H), 1.90 (m, 4H), 2.69 (d, 1H, J = 7.3 Hz, OH), 4.05 (m, 1H), 4.45 (m, 2H), 4.58 (s, 2H), 5.05 (s, 1H), 5.07 (s, 1H), 7.33 (m, 5H); ^{13}C NMR ($CDCl_3$) δ -5.1 (q), 18.0 (s), 25.7 (q), 40.7 (t), 40.9 (t), 70.4 (d and t, 5overlapped), 70.8 (d), 71.3 (d), 107.1 (t), 127.5 (d), 128.4 (d), 138.7 (s), 150.7 (s).

(1*R*,2*S*,3*S*,5*R*)-5-Benzyloxy-3-(*tert*-butyldimethylsilyloxy)-2-hydroxymethyl-cyclohexanol (7c) and its epimer 8c

To a solution of _0 (10.8 g, 31.03 mmol) in dry THF (80 mL) at 0 °C under N_2 was added slowly a solution of 9-BBN in THF (0.5 M, 155 mL, 77.58 mmol). The reaction mixture was slowly warmed up to rt overnight. The reaction was cooled to 0 °C and treated sequentially with EtOH (30 mL), a 2 N NaOH solution (60 mL) and a 35% H_2O_2 solution (60 mL) under stirring. The resulting mixture was stirred at rt for 24 h, then poured into a mixture of EtOAc (300 mL) and H_2O (300 mL). The layers were separated and the aqueous layer was extracted with EtOAc (3x). The combined organic layers were washed with H_2O and brine, dried over Na_2SO_4 and concentrated. The crude product was separated on silica gel (*n*-hexane-EtOAc 5:1, then 1:1) to yield 7c (8.4 g, 74%) and epimer 8c (2.28 g, 20%) as a light-yellow oils.

7c: ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.09 (2s, 6H), 0.91 (s, 9H), 1.52 (ddd, 1H, J = 13.1, 10.1, 2.8 Hz), 1.54 (ddd, 1H, J = 13.1, 10.1, 3.1 Hz), 1.69 (tdd, 1H, J = 10.0, 7.5, 4.1 Hz), 2.10 (dt, 1H, J = 13.1, 4.1 Hz), 2.16 (dt, 1H, J = 13.1, 4.1 Hz), 2.71 (s, 1H), 3.11 (s, 1H), 3.78 (dd, 1H, J = 10.1, 7.5 Hz), 3.85 (td, 1H, J = 10.0, 4.2 Hz), 3.86 (m, 1H), 3.97 (br-td, 1H, J = 10.1, 4.1 Hz), 4.04 (br-dd, 1H, J = 10.1, 4.1 Hz), 4.51 (s, 2H), 7.26-7.37 (m, 5H); ^{13}C NMR ($CDCl_3$) δ -5.0 (q), -4.3 (q), 17.8 (s), 25.7 (q), 38.1 (t), 38.4 (t), 53.2 (d), 63.4 (t), 68.0 (d), 69.4 (d), 70.3 (t), 72.4 (d), 127.4 (d), 127.6 (d), 128.4 (d), 138.7 (s); LISMS (THGLY): 367 $(M+H)^+$; HRMS calcd for $C_{20}H_{35}O_4Si$ $(M+H)^+$ 367.2305, found 367.2341.

8c: ^{1}H NMR ($CDCl_3$) δ 0.07 (s, 3H), 0.08 (s, 3H), 0.85 (s, 9H), 1.40-1.87 (m, 3H), 2.25 (dm, 1H, *J* = 13.2 Hz), 2.48 (dm, 1H, *J* = 13.2 Hz), 3.69 - 4.20 (m, 6H), 4.33 (m, 1H), 4.53 (d, 1H, *J* = 11.7 Hz), 4.62 (d, 1H, *J* = 11.7 Hz), 57.33 (m, 5H), 8.79 (s, 1H); ^{13}C NMR ($CDCl_3$) δ -5.6 (q), -5.0 (q), 21.9 (s), 25.5 (q), 39.2 (2t, overlapped), 45.9 (d), 61.3 (t), 69.0 (d), 69.4 (d), 70.4 (t), 70.8 (d), 127.6 (d), 127.7 (d), 128.4 (d), 138.6 (s); LISMS (THYLY): 367 $(M+H)^+$; HRMS calcd for $C_{20}H_{35}O_4Si$ $(M+H)^+$ 10367.2305, found 367.2335.

(1*R*,2*R*,3*S*,5*S*)-5-Benzyloxy-3-(*tert*-butyldimethylsilyloxy)-2-(*tert*-butyldimethylsilyloxy methyl)cyclohexanol (29).

To a solution of 7c (2.5 g, 6.83 mmol) in DMF 15(50 mL) at rt were added imidazole (930 mg, 13.66 mmol) and TBDMSCl (1.23 g, 8.2 mmol) in portions. The reaction was stirred at rt overnight and quenched with ice. The resulting mixture was evaporated to remove DMF and the residue was partitioned between EtOAc and H_2O. The layers 20were separated and the aqueous layer was extracted with EtOAc (2x). The combined organic layers were washed with H_2O and brine, dried over Na_2SO_4 and concentrated. The residue was chromatographed on silica gel (*n*-hexane-EtOAc 5:1) to yield 29 (2.28 g, 70%) as a light-yellow oil: ^{1}H 25NMR ($CDCl_3$) δ 0.05, 0.06, 0.09 (3s, 12H), 0.89, 0.91 (2s, 18H), 1.53 (m, 2H), 1.72 (qd, 1H, *J* = 9.5, 4.4 Hz), 2.11 (m, 2H), 3.67 (t, 1H, *J* = 9.5 Hz), 3.78 (td, 1H, *J* = 9.5, 4.4 Hz), 3.87 (m, 1H), 4.01 (m, 1H), 4.16 (dd, 1H, *J* = 9.5, 4.4 Hz), 4.46 (d, 1H, *J* = 15.2 Hz), 4.48 (d, 1H, *J* = 3015.2 Hz), 7.33 (m, 5H); ^{13}C NMR ($CDCl_3$) δ -5.7 (q), -5.1 (q), -4.3 (q), 17.8, 18.0 (2s), 25.7 (2q), 37.0 (t), 38.4 (t), 52.2 (d), 66.2 (t), 67.2 (d), 70.1 (t and d overlapped), 72.4 (d), 127.3 (d), 127.4 (d), 128.4 (d), 138.9 (s); LISMS (GLY): 481 $(M+H)^+$; HRMS cald for 35$C_{26}H_{49}O_4Si_2$ $(M+H)^+$ 481.3169, found 481.3199.

(1*R*,2*R*,3*S*,5*S*)-5-Benzyloxy-2-(*tert*-

butyldimethylsilyloxymethyl)-3-(*tert*-butyldimethylsilyoxy)-1-methanesulfonyloxy-cyclohexane (30)

To a solution of 29 (5.4 g, 11.25 mmol) in CH_2Cl_2 (120 mL) at 0 °C was added triethylamine (7.8 mL, 556.25 mmol), followed by dropwise addition of MsCl (1.3 mL, 16.87 mmol). The reaction was stirred at 0 °C for 1h and treated with ice. The resulting mixture was separated and the aqueous layer was extracted with CH_2Cl_2 (2x). The combined organic layers were washed with a diluted HCl solution, H_2O and brine, dried over Na_2SO_4 and concentrated. The residue was chromatographed on silica gel (*n*-hexane-EtOAc 5:1) to afford 30 (5.81 g, 92%) as a white solid: mp 100-101 °C; ^1H NMR ($CDCl_3$) δ 0.08 (2s, 12H), 0.89 (s, 9H), 0.90 (s, 9H), 1.43 (ddd, 1H, *J* = 13.9, 10.0, 2.8 Hz), 1.62 (tt, 1H, *J* = 10.2, 2.0 Hz), 1.71 (ddd, 1H, *J* = 12.8, 10.6, 2.2 Hz), 2.24 (br-d, 1H, *J* = 13.9 Hz), 2.69 (br-d, 1H, *J* = 12.8 Hz), 3.01 (s, 3H), 3.74 (dd, 1H, *J* = 9.9, 2.2 Hz), 3.89 (m, 1H), 3.91 (dd, 1H, *J* = 9.9, 1.8 Hz), 4.19 (td, 1H, *J* = 10.0, 4.7 Hz), 4.45 (d, 1H, *J* = 12.0 Hz), 4.57 (d, 1H, *J* = 12.0 Hz), 5.13 (td, 1H, *J* = 10.6, 4.8 Hz), 7.33 (m, 5H); ^{13}C NMR ($CDCl_3$) δ -5.6 (q), -5.3 (q), -4.6 (q), -3.7 (q), 17.9 (s), 25.8 (q), 35.5 (t), 38.5 (t), 38.8 (q), 51.8 (d), 56.9 (t), 65.1 (d), 70.1 (t), 72.0 (d), 77.5 (d), 127.4 (d), 128.4 (d), 138.5 (s); LISMS(GLY/NBA) 559 $(M+H)^+$; HRMS calcd for $C_{27}H_{51}O_6SSi_2$ $(M+H)^+$ 559.2945, found 559.2979; Anal. Calcd for $C_{27}H_{51}O_6SSi_2$: C 58.02, H 9.02; Found: C 57.96, H 8.82.

(1*S*,3*R*,4*R*,5*S*)-4-*tert*-Butyldimethylsilyloxymethyl-5-*tert*-butyldimethylsilyloxy-3-methanesulfonyloxy-cyclohexanol (31)

A mixture of 30 (3.5 g, 6.27 mmol), Pd/C (10 %, 4.4 g) and $HCOONH_4$ (2.2 g) in MeOH (100 mL) was refluxed and 2 x 1.1 g of $HCOONH_4$ were added every 3 h interval. The reaction was refluxed until all the starting material was consumed (total 14 h). After cooling to rt, the

reaction mixture was filtered through Celite® and the residue was washed with CH_2Cl_2 (3x). The filtrate was concentrated to afford crude 31 (2,83 g, 97%) as a white solid, which was used as such for the next step: mp 135-137 °C; ^1H NMR ($CDCl_3$) δ 0.08, 0.09 (2s, 12H), 0.89 (s, 9H), 0.92 (s, 9H), 1.43 - 1.68 (m, 3H), 1.83 (ddd, 1H, *J* = 13.2, 10.6, 2.8 Hz), 2.07 (br-d, 1H, *J* = 13.2 Hz), 2.44 (br-d, 1H, *J* = 13.2 Hz), 3.02 (s, 3H), 3.72 (dd, 1H, *J* = 10.0, 2.4 Hz), 3.90 (dd, 1H, *J* = 10.0, 2.4 Hz), 4.19 (td, 1H, *J* = 10.6, 4.1 Hz), 4.26 (m, 1H), 5.14 (td, 1H, *J* = 10.6, 4.7 Hz); ^{13}C NMR ($CDCl_3$) δ -5.6 (q), -5.3 (q), -4.7 (q), -3.8 (q), 17.9 (s), 25.8 (q), 38.8 (q), 38.9 (t), 40.8 (t), 51.7 (d), 57.1 (t), 64.9 (d), 65.5 (d), 77.3 (d); LISMS (GLY/NBA) 469 $(M+H)^+$; HRMS calcd for $C_{20}H_{45}O_6SSi_2$ $(M+H)^+$ 469.2475, found 469.2453; Anal. Calcd for $C_{20}H_{45}O_6SSi_2$: C 51.24, H 9.46; Found: C 51.24, H 9.36.

(4*R*,5*S*)-4-*tert*-Butyldimethylsilyloxymethyl-5-tert-butyldimethylsilyloxy-cyclohex-2-en-1-one (33)

A mixture of crude 31 (2.83 g, 6.27 mmol) and MnO_2 (13.6 g, 156.8 mmol) in dry CH_2Cl_2 (100 mL) was stirred vigorously at rt for 21 h. The reaction mixture was filtered through Celite® and washed with CH_2Cl_2. The filtrate was concentrated and the residue was chromatoghraphed on silica gel (*n*-hexane-EtOAc 5:1, then 1:2) to yield starting material 30 (1.56 g, 53 %) and enone 33 (920 mg, 40% over two steps) as a light-yellow oil (solid upon storing in the refrigerator): ^1H NMR ($CDCl_3$) δ 0.07 (s, 12H), 0.89 (s, 18H), 2.50 (m, 1H), 2.46 (dd, 1H, *J* = 16.1, 10.6 Hz), 2.72 (dd, 1H, *J* = 16.1, 4.8 Hz), 3.73 (dd, 1H, *J* = 9.9, 5.6 Hz), 3.85 (dd, 1H, *J* = 9.9, 4.4 Hz), 4.09 (ddd, 1H, *J* = 10.6, 8.1, 4.8 Hz), 6.06 (dd, 1H, *J* = 10.2, 2.6 Hz), 6.88 (dd, 1H, *J* = 10.2, 2.6 Hz); ^{13}C NMR ($CDCl_3$) δ -5.6 (q), -5.5 (q), -5.1 (q), -4.4 (q), 17.8 (s), 18.2 (s), 25.6 (q), 25.8 (q), 47.1 (t), 48.0 (d), 61.8 (t), 68.0 (d), 130.2 (d), 150.6 (d), 199.0 (s); LISMS (THGLY/NBA) 371 $(M+H)^+$; HRMS calcd for

$C_{19}H_{39}O_3Si_2$ $(M+H)^+$ 371.2438, found 371.2432.

(1R,4*R*,5*S*)-5-(tert-Butyldimethylsilyloxy)-4-(tert-butyldimethylsilyloxymethyl)-cyclohex-2-en-1-ol (34)

To a solution of 33 (920 mg, 2.49 mmol) in MeOH (35 mL) at rt under N_2 was added $CeCl_3.7H_2O$ (1.39 g, 3.73 mmol). The mixture was stirred for 0.5 h and a clear solution was obtained. $NaBH_4$ (113 mg, 2.99 mmol) was added in portions and H_2 evolved. The reaction mixture was stirred for 1 h and quenched with H_2O. The mixture was stirred for 15 min and concentrated. The residue was diluted with EtOAc, washed with H_2O and brine, dried over Na_2SO_4 and concentrated. The residue was chromatographed on silica gel (*n*-hexane-EtOAc 10:1) to give 34 (844 mg, 1591%) as a colourless oil: ^1H NMR (500 MHz, $CDCl_3$) δ 0.04 (s, 3H), 0.05 (s, 3H), 0.10 (s, 3H), 0.11 (s, 3H), 0.89 (s, 9H), 0.90 (s, 9H), 1.94 (ddd, 1H, J = 13.7, 5.3, 3.9 Hz), 1.99 (ddd, 1H, J = 13.7, 4.5, 2.6 Hz), 2.36 (m, 1H), 2.94 (d, 1H, J = 9.8 Hz), 3.38 (dd, 1H, J = 10.1, 7.8 Hz), 3.56 (dd, 1H, J = 10.1, 5.0 Hz), 4.09 (pseudo sext, 1H, J = 9.8, 4.5, 4.0, 3.9 Hz), 4.20 (pseudo pent, 1H, J = 5.3, 3.4, 2.6 Hz), 5.61 (dd, 1H, J = 10.0, 3.9 Hz), 5.95 (ddd, 1H, J = 10.0, 4.0, 1.8 Hz); ^{13}C NMR ($CDCl_3$) δ -5.5 (q), -5.4 (q), -4.9 (q), -4.8 (q), 18.0 (s), 18.3 (s), 25.8 (q), 25.9 (q), 35.6 (t), 46.5 (d), 63.5 (t), 64.8 (d), 67.7 (d), 127.0 (d), 131.1 (d); LISMS (THGLY/NBA) 373 $(M+H)^+$; HRMS calcd for $C_{19}H_{40}O_3Si_2$ $(M+H)^+$ 373.2594, found 373.2626; Anal. Calcd for $C_{19}H_{40}O_3Si_2$: C 61.23, H 10.82; Found: C 61.34, H 10.83.

9-[(1*S*,4*R*,5*S*)-5-(tert-Butyldimethylsilyloxy)-4-(tert-butyldimethylsilyloxymethyl)-2-cyclohexenyl]adenine (35a)

To a mixture of 34 (660 mg, 1.774 mmol), adenine (480 mg, 3.55 mmol) and PPh_3 (931 mg, 3.55 mmol) in dry dioxane (20 mL) under N_2 at rt was added a solution of DEAD (565 µL, 3.55 mmol) in dry dioxane (10 mL) over a period of 45 min. The reaction mixture was stirred at rt

overnight, concentrated and the residue was chromatographed on silica gel (CH_2Cl_2-MeOH 50:1, then 20:1) to yield crude 35a (960 mg) as a yellow foam: ^1H NMR ($CDCl_3$) δ -0.12 (s, 3H), -0.06 (s, 3H), 0.10 (s, 3H), 0.11 (s, 3H), 0.83 (s, 9H), 0.94 (s, 9H), 2.01 - 2.25 (m, 2H), 2.32 (m, 1H), 3.73 (dd, 1H, J = 9.9, 4.8 Hz), 3.82 (dd, 1H, J = 9.9, 4.4 Hz), 3.97 (ddd, 1H, J = 10.2, 7.0, 4.0 Hz), 5.37 (m, 1H), 5.73 (s, 2H), 5.88 (ddd, 1H, J = 9.9, 3.7, 2.5 Hz), 6.06 (ddd, 1H, J = 9.9, 2.2, 1.1 Hz), 7.86 (s, 1H), 8.39 (s, 1H); ^{13}C NMR ($CDCl_3$) δ -5.5 (q), -5.4 (q), -5.0 (q), -4.6 (q), 17.8 (s), 18.3 (s), 25.6 (q), 25.9 (q), 36.5 (t), 47.2 (d), 49.6 (d), 62.9 (t), 64.5 (d), 120.2 (s), 124.4 (d), 134.9 (d), 139.9 (d), 149.8 (s), 153.0 (d), 155.5 (s); LISMS (THGLY/NBA) 490 $(M+H)^+$; HRMS calcd for $C_{24}H_{44}N_5O_2Si_2$ $(M+H)^+$ 490.3034, found 490.3058.

9-[(1*S*,4*R*,5*S*)-5-Hydroxy-4-hydroxymethyl-2-cyclohexenyl]adenine (36)

Crude 35a was treated with TFA-H_2O (3:1, 40 mL) at rt overnight. The reaction mixture was concentrated and co-evaporated with toluene (2x). The residue was chromatographed on silica gel (CH_2Cl_2-MeOH 20:1, then 5:1) to afford 36 (149 mg, 54% over two steps): Mp 90 - 92 °C; ^1H NMR (CD_3OD) δ 2.01-2.33 (m, 3H), 3.80 (d, 2H, J = 4.8 Hz), 3.84 (m, 1H), 5.33 (m, 1H), 5.94 (ddd, 1H, J = 9.9, 3.7, 2.6 Hz), 6.13 (ddd, 1H, J = 9.9, 2.5, 1.4 Hz), 8.09 (s, 1H), 8.21 (s, 1H); ^{13}C NMR (CD_3OD) δ 37.3 (t), 47.9 (d), 51.1 (d), 63.1 (t), 64.7 (d), 120.6 (s), 125.3 (d), 136.1 (d), 141.6 (d), 150.4 (s), 153.7 (d), 157.5 (s); UV λ_{max} (MeOH) = 260 nm; LISMS (THGLY/NBA) 262 $(M+H)^+$; HRMS calcd for $C_{12}H_{16}N_5O_2$ $(M+H)^+$ 262.1304, found 262.1359; Anal. Calcd for $C_{12}H_{16}N_5O_2.0.7H_2O$: C 52.62, H 6.04, N 25.57; Found: C 52.62, H5.95, N 25.77.

9-[(1*R*,3*S*,4*R*)-3-Hydroxy-4-hydroxymethyl-cyclohexanyl]adenine (2a)

A mixture of 36 (45 mg, 0.17 mmol) and Pd/C (10%, 40 mg) in MeOH (5 mL) was stirred under H_2 at rt for 24 h. The reaction mixture was cooled to rt and filtered through Celite® and washed with MeOH. The filtrate was concentrated and theireyidue was purified by reversed-phase HPLC (5% CH_3CN in H_2O) to yield 2a (35 mg, 78%) as a white foam: ^1H NMR (CD_3OD) δ 1.71 (m, 1H), 1.87-2.18 (m, 5H), 2.39 (m, 1H), 3.69 (dd, 1H, J = 14.0, 7.3 Hz), 3.74 (dd, 1H, J = 14.0, 6.9 Hz), 4.12 (m, 1H), 4.87 (m, 1H, overlapped with HOD), 8.18 (s, 1H), 8.21 (s, 1H); ^{13}C NMR (CD_3OD) δ 22.6 (t), 28.7 (t), 36.1 (t), 53.6 (d), 51.9 (d), 63.3 (t), 68.4 (d), 120.4 (s), 141.1 (d), 150.6 (s), 153.5 (d), 157.4 (s); LISMS (THGLY/NBA) 264 $(M+H)^+$; HRMS calcd for $C_{12}H_{18}N_5O_2$ $(M+H)^+$ 264.1460, found 264.1449.

Figure 1. Mechanism of Pd (0) coupling reaction which may yield the desired compound C.

Figure 2. ^1H NMR experiment demonstrates the solvent-dependent conformational equilibrium of compound 34.

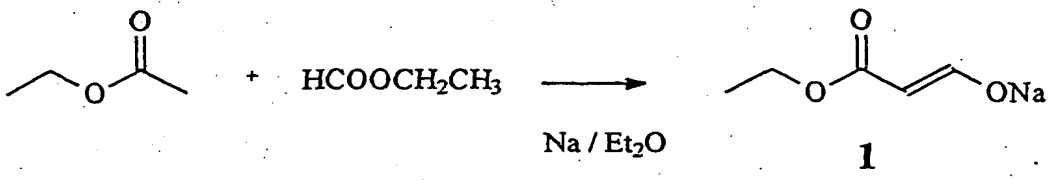
Figure 8 (a) TBDMSCl (1.2 eq), imidazole (2 eq), DMF, r.t., 48% starting from 6a; (b) MsCl, Et_3N, CH_2Cl_2, 0 °C, 93 %; (c) DBU, toluene, or KOH, H_2O / THF; (d) DEAD, PPh_3, THF; (e) I_2, PPh_3, imidazole, toluene, reflux, 34%; (f) DBU, toluene, reflux, 68%.

Figure 9 (a) $(CH_3)_2C(OCH_3)_2$, PPTS, acetone/THF (1:2), r.t., 94%; (b) Pd-C (10%), $HCOONH_4$, MeOH, reflux, 100%; (c) PDC, CH_2Cl_2, r.t., 94%; (d) TBAF, THF, r.t.; (e) KF, 18-Crown-6, THF, r.t., 62% 19; (f) $CeCl_3.7H_2O$, $NaBH_4$, MeOH, 90%; (g) PPh_3, DEAD, AcOH, THF; (h) Ac_2O, DMAP, CH_2Cl_2, 0 °C, 95%; (i) Adenine, NaH, $Pd(PPh_3)_4$, DMF/THF; (j) PPTS, MeOH, r.t., 59%; (k) Bz_2O, DMAP, CH_2Cl_2, 0 °C, 95%.

Figure 10 (a) TBDMSCl (1.2 eq), imidazole (1.5 eq), DMF, r.t., 70%; (b) MsCl, Et_3N, CH_2Cl_2, 0 °C, 100%;

(c) Pd-C(10%), $HCOONH_4$, MeOH, reflux, 76%; (d) MnO_2, CH_2Cl_2, r.t., 48% and 47% recovery of 31; (e) $NaBH_4$, $CeCl_3.7H_2O$, MeOH, 0 °C → r.t., 100%.

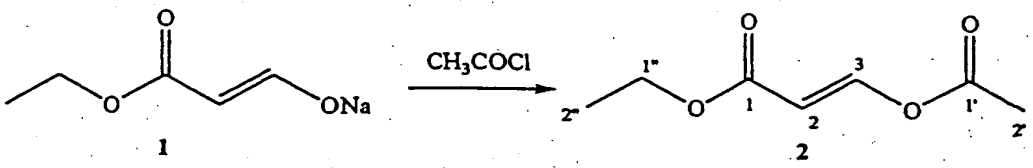
Sodium salt of Ethyl-β-hydroxyacrylate sodium salt (1)



1

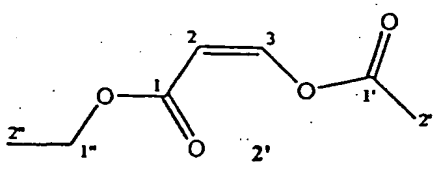
In a 1 L three necked flask, under inert atmosphere and equipped with an addition funnel, a well stirred suspension of fresh sodium pieces (23.0 g, 1.0 mol) in dry diethyl ether (400 mL) was prepared. A mixture of ethyl acetate (88.0 g, 1.0 mol) and ethyl formate (74.0 g, 1.0 mol) was added dropwise over a period of 45 minutes. Stirring was continued for an additional 14 hours using an ice bath, avoiding the reaction to become too vigorous. The resulting suspension was kept in the refrigerator for 8 hours, after which it was filtered, washed with dry diethyl ether (100 mL) and dried *in vacuo* to obtain 1 as a pale yellow solid (85.0 g, 61% yield).

Ethyl β-acetoxyacrylate [cis (2') + trans (2)]



$C_7H_{10}O_4$
Exact Mass: 158.1
Mol.Wt.: 158.2
C, 53.1; H, 6.37; O, 40.47

EPO - DG 1
07. 12. 2000
(54)



In a 2 L flask on an ice-bath, under an inert atmosphere, a well stirred suspension of the sodium salt 1 (85.0 g, 616 mmol) was prepared in dry diethyl ether (850 mL), to which acetyl chloride (52.9 mL, 58.2 g, 739 mmol) was added dropwise over 15 minutes. The mixture was stirred for an additional 6 hours, after which it was neutralized with a saturated aqueous solution of $NaHCO_3$ (250 mL). Both phases were separated and the aqueous phase was extracted with diethyl ether (5 x 200 mL). The combined organic phases were dried over Na_2SO_4, filtered and evaporated *in vacuo* to obtain a residual red oil (59.1 g). Distillation *in vacuo* (70 °C, 1 Torr aprox.) afforded a mixture of 2 and 2', as a pure colorless oil (36.5 g, 23 % yield in two steps) with a *cis / trans* proportion of 4:10 (^{1}H-NMR).

analytical data of 2 (*trans* isomer)

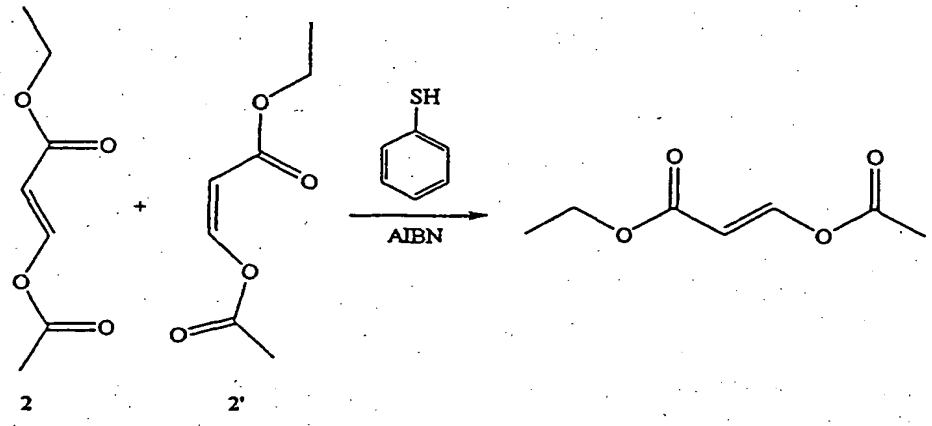
^{1}H-NMR (200 MHz, $CDCl_3$) δ: 1.30 (t, J = 7.2 Hz, 3H, 2"-H), 2.22 (s, 3H, 2'-H), 4.21 (q, J = 7.2 Hz, 2H, 1"-H), 5.72 (d, J = 12.6 Hz, 1H, 2-H), 8.30 (d, J = 12.6 Hz, 1 H, 3-

H).

analytical data of 2' (*cis* isomer)

^{1}H-NMR (200 MHz, $CDCl_3$) δ: 1.30 (t, *J* = 7.2 Hz, 3H, 2"-H), 2.28 (s, 3H, 2'-H), 4.20 (q, *J* = 7.4 Hz, 2H, 1"-H), 5.30 (d, *J* = 7.3 Hz, 1H, 2-H), 7.54 (d, *J* = 7.3 Hz, 1H, 3-H).

Isomerization of the *cis/trans* mixture (2/2') to ethyl *trans*-β-acetoxyacrylate (2)

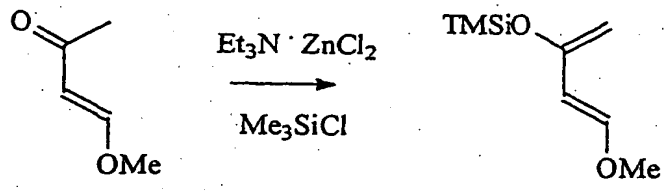


2 2'

In a well-closed flask, under magnetic stirring, the 2/2' mixture obtained from several operations (52.5 g, 39:100 *cis* / *trans* proportion, 332 mmol) was treated with thiophenol (16.3 ml, 17.5 g, 159 mmol) and 2,2'-azobis(2-methylpropionitrile) (AIBN, 8.31 g, 50.6 mmol) and the mixture was heated to 80 °C for 2.5 hours. The flask was cooled for 2 hours and the crude was diluted with ethyl acetate (400 mL) and washed with an aqueous solution of NaOH 0.01 N (400 mL). The organics were dried over Na_2SO_4, filtered and evaporated *in vacuo* to leave a pale yellow oil. Distillation *in vacuo* (53 °C, 0.5-1.0 Torr) afforded 2 (55.8 g, quantitative yield) with a *cis* / *trans* proportion of 3:97 (^{1}H-NMR), slightly

contaminated with aromatic sulphurated products.

Preparation of (*E*)-1-methoxy-3-trimethylsilyloxy-1,3-butadiene (4)



3 4

Under an inert atmosphere anhydrous $ZnCl_2$ (2.52 g, 18.5 mmol) was slowly added under magnetic stirring to triethylamine (distilled over KOH) (145 g, 200 mL, 1.43 mol). The mixture was stirred for 1 hour at room temperature until a fine suspension was obtained. A solution of compound 3 (63.1 g, 630 mmol) in toluene (190 mL) was then added over 5 min, followed by gradual addition of chlorotrimethylsilane (137.0 g, 160 mL, 1.26 mol) over a period of 10 min. An exothermic reaction was noted. After 30 minutes, the temperature was raised to 40 °C and stirring was continued overnight. Following cooling, the reaction mixture was diluted with diethyl ether (1 L), filtered and washed with diethyl ether (4 x 100 mL). The combined filtrate and ether washings were concentrated *in vacuo* to leave a brown oil. Distillation trough a Vigreux column (52 °C, 1.0 Torr) afforded compound 4 in a middle cut, slightly contaminated with compound 3 [80.1 g, 91% purity (^{1}H-NMR), 67% yield of 4].

NOTE: compound 4 is commercially available (e.g. Aldrich®).

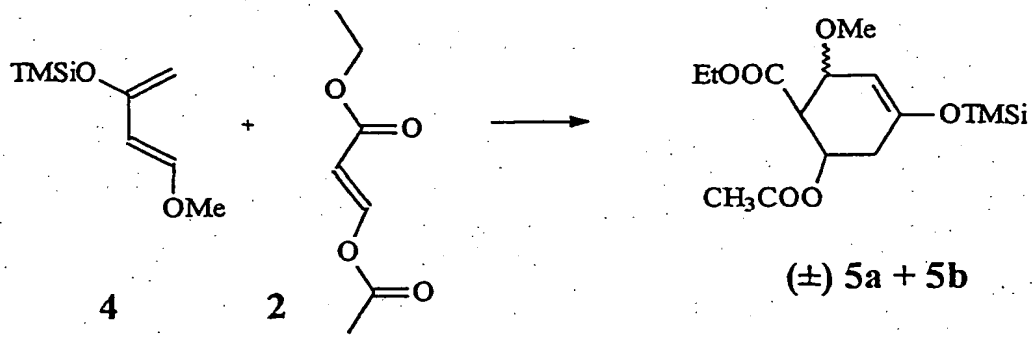
analytical data of 4.

^{1}H-NMR (200 MHz, $CDCl_3$) δ: 0.23 [s, 9H, $OSi(C\underline{H}_3)_3$], 3.59 (s, 3H, $OC\underline{H}_3$), 4.09 (d, *J* = 8.2 Hz, 2H, 4-H), 5.35 (d, *J* = 512.2 Hz, 1H, 2-H), 6.83 (d, *J* = 12.2 Hz, 1H, 3-H).

Diels-Alder adduct of 2 and 4:

5-*O*-acetyl-4-ethoxycarbonyl-3-*O*-methyl-1-*O*-trimethylsilyl-cyclohexen-1,3,5-triol

[(±) 5a + 5b]



4 2 (±) 5a + 5b

In a 250 mL round bottom flask a small amount of hydroquinone (372 mg) was added under magnetic stirring to a mixture of the Danishefky diene [4, 72.9 g, 91% purity (^{1}H-NMR), 385 mmol] and 2 (55.8 g, *cis/trans* 3:97, 353 mmol) and the mixture was heated at 180 °C for 201.5 hours. An additional amount of 372 mg of hydroquinone was added and the reaction mixture was distilled *in vacuo* (94 °C, 3.0 x 10^{-2} mm Hg) to afford a slightly contaminated mixture of (±) 5a + 5b (72.0 g, 62% yield), with the substituents at the 4- and 5-position oriented

in *trans*.

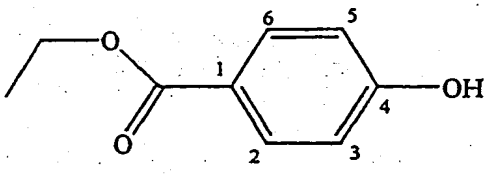
NOTE: Upon increasing the temperature of the distillation bath to 170 °C or higher, different quantities of the phenolic derivative 6 are obtained. The phenol derivative 6 likewise is obtained as the main isolated product when purification on silica gel is undertaken. The addition of fresh hydroquinone right before the distillation seems to avoid the formation of 106. Compound 6 could not detected by NMR when using this improved procedure.

representative analytical data for the major derivative (±) 5a (substituents at 3 and 4 in trans)

^{1}H NMR ($CDCl_3$) δ 0.21 (s, 9H), 1.27 (t, 3H, J = 7.3 Hz), 2.01 (s, 3H), 2.19 (m, 1H), 2.55 (dd, 1H, J = 16.7, 5.5 Hz), 2.77 (dd, 1H, J = 11.4, 8.4 Hz), 3.31 (s, 3H), 4.20 (m, 2H), 4.35 (dm, 1H, J = 8.4 Hz), 4.94 (t, 1H, J = 2.2 Hz), 5.13 (ddd, 1H, J = 11.0, 9.2, 5.9 Hz).

^{13}C NMR ($CDCl_3$) δ 0.06 (q), 14.2(q), 20.8 (q), 35.4 (t), 51.1 (t), 55.4 (q), 60.9 (t), 68.8 (d), 76.5 (d), 103.3 (d), 149.3 (s), 170.0 (s), 172.2 (s).

Ethyl p-hydroxybenzoate (6) : analytical data

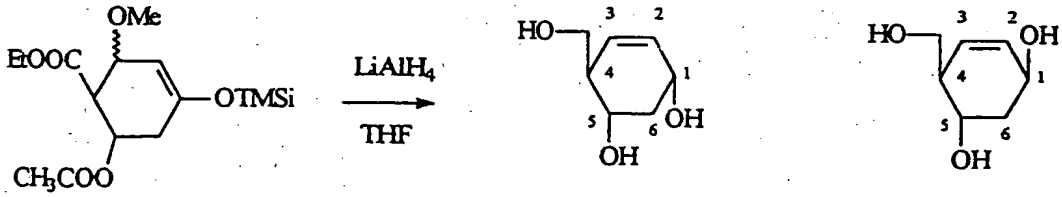


6

^{1}H-NMR (200 MHz, $CDCl_3$) δ: 1.39 (t, J = 7.2 Hz, 3H, -5$CH_2\underline{CH_3}$), 4.37 (q, 2H, -$\underline{CH_2}CH_3$), 6.91 [d, J = 8.9 Hz, 2H, 3(5)-H], 7.36 (broad s, 1H, 4-$\underline{OH}$), 7.96 [d, J = 8.9 Hz, 2H, 2(6)-H].

^{13}C-NMR (50.3 MHz, $CDCl_3$) δ: 14.1 (-$CH_2\underline{CH_3}$), 61.1 (-$\underline{CH_2}CH_3$), 115.3 [C3(5)], 122.2 (C1), 132.0 [C2(6)], 160.7 (C4), 10167.6 (C=O).

(±) 4-hydroxymethyl-cyclohex-2-en-1,5-diol (7a)



(±) 5 (±) 7a (±) 7

15

In a 1L three necked bottom flask on a ice-NaCl bath, a suspension of $LiAlH_4$ (25.0 g, 658 mmol) in dry THF (220 mL) was prepared under magnetic stirring in an inert atmosphere. To this cooled suspension, a solution of the 20impure mixture of 5a + 5b (27.2 g) in dry THF (85 mL) was added dropwise during 30 minutes. After stirring at 0 °C

for 2 hours, the reaction was continued at room temperature for an additional 19 hours. The mixture became very viscous and was diluted with dry THF (110 mL). After cooling on an ice-NaCl bath, the mixture was treated consecutively and very carefully (equipping the system with a good gas-exit) with water (25 mL), stirring for 15 minutes, with 15% aqueous NaOH (25 ml), stirring for 15 minutes more, and finally with more water (75 ml). A dry granular precipitate was produced, which was easy to filter and wash. The suspension was stirred for 30 minutes and the precipitate was filtered over a layer of Celite®, and washed with water (5 x 100 mL) and ethyl acetate (3 x 100 mL). Both phases were separated and the aqueous phase was washed with ethyl acetate (3 x 100 mL). The aqueous phase was evaporated to dryness to give a brown gummy residue (21.1 g) which was filtered through a silica gel column (210 g) packed with ethyl acetate, eluting with mixtures of EtOAc/MeOH of increasing polarity. The title product 7a was isolated as a pale yellow oil (3.55g, 24.7 mmol, 30%), preceded by its epimer 7b (6.44g) as an impure mixture.

analytical data of 7a

^{1}H NMR ($CDCl_3$ + DMSO-d_6) δ 1.48 (td, 1H, J = 11.3, 9.2 Hz), 2.02 - 2.23 (m, 2H), 3.35 (m, 1H), 3.61 (m, 2H), 3.75 (d, 1H, J = 5.8 Hz, OH), 4.01 (t, 1H, J = 4.6 Hz, OH), 4.11 (m, 1H), 4.20 (d, 1H, J = 3.3 Hz, OH), 5.25 (dt, 1H, J = 9.9, 2.0 Hz), 5.58 (dm, 1H, J = 9.9 Hz).

^{13}C NMR ($CDCl_3$ + DMSO-d_6) δ 39.7 (t), 45.8 (d), 65.2 (t), 65.9 (d), 69.4 (d), 126.1 (d), 132.4 (d).

LISMS (THGLY/NaOAc) 167 $(M+Na)^+$ ($C_7H_{12}O_3$)

Data of 7b:

^{1}H NMR (DMSO-d_6) δ 1.37 (td, 1H, J = 11.7, 9.9 Hz), 1.92 - 2.10 (m, 2H), 3.24 - 3.45 (m, 2H), 3.63 (dt, 1H, J =

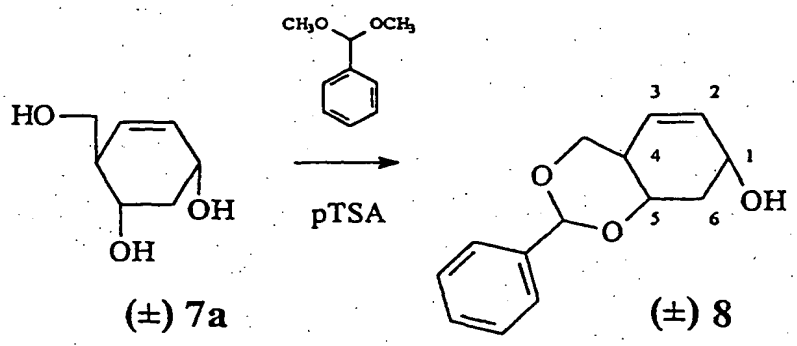
10.2, 4.4 Hz), 4.07 (m, 1H), 4.49 (t, 1H, J = 5.3 Hz, OH), 4.63 (d, 1H, J = 5.1 Hz, OH), 4.70 (d, 1H, J = 5.9 Hz, OH), 5.52 (d, 1H, J = 11.0 Hz), 5.57 (d, 1H, J = 11.0 Hz).

^{13}C NMR (DMSO-d_6) δ 42.0 (t), 47.2 (d), 62.2 (t), 65.9 (d), 66.3 (d), 127.7 (d), 132.8 (d).

LISMS (THGLY/TFA) 145 $(M+H)^+$ ($C_7H_{12}O_3$)

Formation of the ketal of 7a:

(±) 5,7-*O*-benzylidene-4-hydroxymethyl-cyclohex-2-en-1,5-diol (8)



(±) 7a (±) 8

Under an inert atmosphere benzaldehyde dimethyl acetal (6.2 mL, 41.2 mmol) and *p*-toluenesulfonic acid monohydrate (300 mg, 1.58 mmol) were added to a solution of (±)7a (4.49 g, 31.1 mmol) in dry dioxane (140 mL). The mixture was stirred at room temperature for 24 hours and subsequently poured into ethyl acetate (100 mL), washed with water (230 mL), dried over Na_2SO_4 and concentrated to give a white residue (8.91 g). Chromatographic purification on silica gel (270 g) eluting with mixtures of hexane/EtOAc of increasing polarity afforded the desired product 8 as a white crystalline solid (5.06 g,

70% yield, 80% yield based on recovered 7a).

The aqueous phase was evaporated to dryness, to recover the starting material 7a (600 mg, 13 % recovery).

analytical data of 8a

^{1}H-NMR (500 MHz, $CDCl_3$) δ: 1.60 (d, J = 7.3 Hz, 1H, OH), 1.80 (ddd, 1H, 6-H_a), 2.53 (ddd, 1H, 6-H_e), 2.60 (m, 1H, 4-H), 3.61 (t, J = 11.2 Hz, 1H, 7-H_a), 3.68 (ddd, 1H, 5-H), 4.26 (dd, J = 10.7 and 4.4 Hz, 1H, 7-H_e), 4.53 (m, 1H, H-1), 5.42 (ddd, J = 9.7 Hz, 1H, 2-H), 5.59 (s, 1H, PhC<u>H</u>), 5.74 (ddd, J = 9.8 Hz, 1H, 3-H), 7.31-7.40 and 7.48-7.53 (m, 5H, arom-H).

^{13}C-NMR (500 MHz, $CDCl_3$) δ: 38.6 (C-6), 40.1 (C-4), 68.0 (C-1), 70.7 (C-7), 77.7 (C-5), 102.2 (PhCH), 125.0 (C-2), 126.2 (ar-C_o), 128.3 (ar-C_m), 129.0 (ar-C_i), 132.7 (C-3), 138.1 (ar-C_p).

LISMS (GLY/TFA) 233 $(M+H)^+$ ($C_{14}H_{16}O_3$)

Additional amounts of the desired 8a can be obtained using the other epimer 7b, using an oxidation - reduction cycle as outlined below.

Therefore, the crude 7b (2.3 g, 14.58 mmol) was treated with benzaldehyde dimethyl acetal (3.28 mL, 21.87 mmol) in the presence of p-toluenesulfonic acid monohydrate (PTSA, 138 mg, 0.73 mmol) in 1,4-dioxane (30 mL) at r.t. for two days. Ice was added and the mixture was stirred at r.t. for 0.5 hr and extracted with EtOAc (3x). The combined organic solvents were washed with water and brine, dried over sodium sulfate and concentrated. The residue was purified on silica gel (hexane /EtOAc 1:1) to afford a mixture of 8b and 8a (3:1, 1.2 g) as a light yellow solid.

The mixture of 8a/8b (3:1, 415 mg, 1.79 mmol) and MnO_2 (1.56 g, 17.9 mmol, 10 eq) in dry CH_2Cl_2 (15 mL)

was stirred at rt for 21 hrs. The reaction mixture was diluted with CH_2Cl_2 and filtered through Celite. The filtrate was concentrated and the residue was chromatographed on silica gel (hexane - EtOAc 2:1) to afford 9 (340 mg, 83%) as a colourless oil.

^{1}H NMR ($CDCl_3$) δ 2.65 (dd, 1H, J = 16.4, 13.1 Hz), 2.83 (m, 1H), 2.95 (dd, 1H, J = 16.4, 4.8 Hz), 3.79 (t, 1H, J = 11.1 Hz), 4.04 (ddd, 1H, J = 13.1, 9.2, 4.8 Hz), 4.45 (dd, 1H, J = 11.1, 4.8 Hz), 5.63 (s, 1H), 6.13 (dd, 1H, J = 9.9, 2.9 Hz), 6.58 (dd, 1H, J = 9.9, 1.8 Hz), 7.39 (m, 3H), 7.51 (m, 2H).

^{13}C NMR ($CDCl_3$) δ 39.9 (d), 44.3 (t), 69.2 (t), 77.4 (d), 101.7 (d), 126.1 (d), 128.4 (d), 129.2 (d), 132.1 (d), 137.5 (s), 144.9 (d), 196.8 (s).

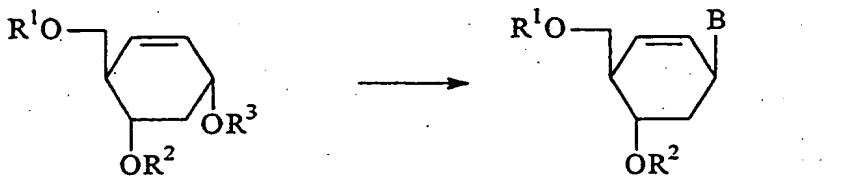
LISMS (NBA) 231 $(M+H)^+$. ($C_{14}H_{14}O_3$)

Conversion of 9 to 8a:

To a solution of 9 (340 mg, 1.5 mmol) in MeOH (15 mL) at rt was addded $CeCl_3.7H_2O$ (838 mg, 2.25 mmol, 1.5 eq). After stirring at rt for 1 hr, $NaBH_4$ (68 mg, 1.8 mmol, 1.2 eq) was added in portions. The reaction was stirred at rt for 2 hrs and quenched with crushed ice. The resulting mixture was stirred at rt for 0.5 hr and concentrated. The residue was taken into ethyl acetate and washed with water and brine, dried over sodium phosphate and concentrated. The residue was chromatographed on silica gel (hexane - EtOAc 5:1 and 1:1) to give 8a as a white solid which proved identical to the previous material.

The product 8a, or its analogues, either under their racemic form, or under the form of their separated isomers, as represented by the general structure III, can be used for synthesis of cyclohexenyl nucleoside

analogues of general structure IV, according to standard procedures for alkylation of heterocyclic bases. Hereto, in the general structure III, R^1 and R^2 are representing protecting groups (e.g. R_1, R_2 = C_6H_5-CH=), and R^3 represents a leaving functionality (e.g. R^3 = SO_2CH_3, SO_2CF_3, $SO_2C_6H_4CH_3$, $SO_2C_6H_4CH_3$, $SO_2C_6H_4Br$) enabling nucleophilic substitution reactions, or R^3 represents hydrogen, to be used in Mitsunobu reactions.



(III) (IV)

example:

N^2-Benzoyl-9-(5-hydroxy-4-hydroxymethyl-2-cyclohexenyl)guanine ((±) 11)

To a mixture of (±) 8a (696 mg, 3 mmol), 2-amino-6-chloropurine (1.02 g, 6 mmol) and triphenyl phosphine (PPh_3, 1.57 g, 6 mmol) in dry 1,4-dioxane (30 mL) was added slowly a solution of DEAD (945 mL, 6 mmol) in dry 1,4-dioxane (10 mL). The reaction was stirred at r.t. overnight and concentrated. The residue was taken on silica gel and chromatographed on silica gel (CH_2Cl_2 / MeOH 100:1 and 50:1) to afford the crude 10 (2 g) and the N_7-epimer (140 mg) as a white solid.

The crude 10 (2 g) was treated with TFA/H_2O (3:1, 20 mL) at r.t. for 2 days. The reaction mixture was

concentrated and coevaporated with toluene. The residue was chromatographed on silica gel (CH_2Cl_2 / MeOH 50:1 and 10:1) to produce (±) 11 (220 mg, 27 % overall yield starting from 8a).

The spectrum of 11 is identical to that previously reported.

Table I

Antiviral activity of D-cyclohexenyl G and L-cyclohexenyl G in comparison with approved antiviral drugs: 50% inhibitory concentration (IC_{50}) values are given in µg/ml.

Virus	D-cyclohexenyl G		L-cyclohexenyl G		Brivudin	Acyclovir	Ganciclovir	Cidofovir
	Activity	Selectivity index	Activity	Selectivity index				
HSV-1 (KOS) [a]	0.002 [b]	> 2.10^5	0.003 [b]	> 5.10^3	0.001 [b]	0.01 [b]	0.001 [b]	ND
HSV-1 (F) [a]	0.002 [b]	> 2.10^5	0.003 [b]	> 5.10^3	0.001 [b]	0.003 [b]	0.001 [b]	ND
HSV-1 (McIntyre) [a]	0.004 [b]	> 1.10^5	0.004 [b]	> 4.10^3	0.001 [b]	0.005 [b]	0.001 [b]	ND
HSV-2 (G) [a]	0.05 [b]	> 8.10^3	0.07 [b]	> $2.2\ 10^2$	> 80 [b]	0.02 [b]	0.002 [b]	ND
HSV-2 (196) [a]	0.07 [b]	> 5.10^3	0.1 [b]	> $1.8\ 10^2$	> 80 [b]	0.02 [b]	0.001 [b]	ND
HSV-2 (Lyons) [a]	0.07 [b]	> 5.10^3	0.07 [b]	> $2.2\ 10^2$	> 80 [b]	0.02 [b]	0.001 [b]	ND
HSV-1 (TK⁻ KOS ACV^r) [a]	0.38 [b]	> 1.10^3	1.28 [b]	> 12	> 80 [b]	0.6 [b]	0.48 [b]	ND
HSV-1 (TK⁻/TK⁺ VMW1837) [a]	0.01 [b]	>4.10^4	0.01 [b]	> $1.8\ 10^3$	> 80 [b]	0.07 [b]	0.01 [b]	ND
VZV (YS) [c]	0.49 [d]	> 40	1.2 [d]	> 16	0.03 [d]	1.1 [d]	ND	ND
VZV (OKA) [c]	0.64 [d]	> 30	1.9 [d]	> 10	0.003 [d]	0.8 [d]	ND	ND
VZV (TK⁻ 07/1) [c]	2.1 [d]	> 10	5.8 [d]	> 3	> 20 [d]	13 [d]	ND	ND
VZV (TK⁻ YS/R) [c]	2.0 [d]	> 7	6.0 [d]	> 3	> 50 [d]	20 [d]	ND	ND
CMV (AD 169) [c]	0.6 [d]	> 30	1.5 [d]	> 13	ND	ND	0.6 [d]	0.08 [d]
CMV (Davis) [c]	0.8 [d]	> 25	1.7 [d]	> 12	ND	ND	0.8 [d]	0.2 [d]

(a) Activity determined in E_6SM cell cultures
(b) Minimum inhibitory concentration (µg/ml) required to reduce virus-induced cytopathogenicity by 50%
(c) Activity determined in HEL cells
(d) Inhibitory concentration (µg/ml) required to reduce virus plaque formation by 50%. Virus input was 20 plaque forming units (PFU)
ND : not determined

1003702791 - 080502

Table II

Cytotoxicity of D-cyclohexenyl G and L-cyclohexenyl G in four different cell lines (concentrations in µg/ml)

Cell line	D-cyclohexenyl G	L-cyclohexenyl G	Brivudin	Acyclovir	Ganciclovir	Cidofovir
HeLa[a]	400	400	≥ 400	ND	ND	ND
Vero[a]	400	400	≥ 400	ND	ND	ND
E_6SM^a	> 400	> 16	≥ 400	≥ 400	> 100	ND
HEL[b]	> 20	> 20	> 50	> 50	> 50	> 50
HEL[c]	11	> 20	> 200	> 200	> 50	> 50

(a) Minimum cytotoxic concentration causing a microscopically detectable alteration of cell morphology

(b) Cytotoxic concentration required to reduce cell growth by 50%

SUBSTITUTE SHEET (RULE 26)